

REMARKS

Claims 1-2 and 4-21 are of record pending in this application. No claims are currently amended or added, but, claims 5, 6, 9-16, 17, 18 and 20 are hereby cancelled. Claims 1, 2, 4, 7, 8, 19 and 21 remain pending and in issue in the application.

Reconsideration of all outstanding rejections for all such claims remaining here in issue, i.e., claims 1, 2, 4, 7, 8, 19 and 21 and re-examination and allowance of all claims 1, 2, 4, 7, 8, 19 and 21 are hereby respectfully requested. The issues of the outstanding Final Office Action, mailed November 5, 2009 (hereafter, “the Final Office Action”), will now be addressed *seriatim*.

The Restriction/Election Requirements

Applicants note that, in response to the Election/Restriction Requirement and the subsequent restatement of the Election/Restriction Requirement in the Final Office Action, Applicants respectfully maintain their election and traverse as set forth in its response of July 8, 2008. Applicants also respectfully note that neither 37 CFR 1.144, nor MPEP 821.01 require cancellation at this stage (no allowable subject matter having been noted); however, Applicants have, nevertheless, and for convenience only, hereby cancelled the claims formerly withdrawn from consideration.

Claim Objections

Claims 7, 9-13 and 14-16 were objected to with respect to informalities. Claims 9-13 and 14-16 have been cancelled. The status identifier of Claim 7 has been changed herein. Applicants respectfully request reconsideration and withdrawal of the claim objections.

Claim Rejections under 35 USC §103(a)

Claims 1, 2, 4, 7-16, 19, and 21 were rejected under 35 USC § 103(a) as purportedly being unpatentable over Moebs et al. (The Plant J. 11(2): 227-236, 1997; hereinafter “Moebs”) in view of Day et al. (FEBS letters 486 (1998); hereinafter “Day”), Arend et al. (Biotechnol. Bioeng. 76(2):126-31, 2001, esp. pp. 129-130; hereinafter “Arend”) and Priefert (Applied Microbiol. Biotechnol. 56:296-314 (2001); hereinafter “Priefert”). Applicants respectfully traverse these rejections for at least the reasons discussed below, and respectfully submit that Applicants’ developments would indeed NOT be obvious to one skilled in the art.

Applicants reincorporate in full the remarks presented in the Office Action Response of August 5, 2009, and respectfully submit that none of the cited references form a proper basis, alone or in any combination, for rejection under 35 USC §103(a). The present application, specification and claims, provides detailed technical information on the introduction of the complete biosynthesis pathways of both vanillin and a glycosyltransferase into a single yeast microorganism and thereby perform the method of the herein presented claim 1. See Declaration of Professor Birger Lindberg Møller, page 2, paragraph 4, lines 3-7, and Declaration of Joergen Hansen, id.

In short, the pending claims require, inter alia, producing both the aglycon vanillin and the corresponding glycosylated form of the aglycon vanillin in a single yeast cell, and then deglycosylating the glycosylated vanillin. Declaration of Birger Møller, page 2, para. 4, inter alia, and Declaration of Joergen Hansen, id. The cited art simply does not put these two elements or operations together in a single yeast cell, not identically, nor by teaching, suggestion or motivation. Møller Declaration, paras. 4, 6, 7, 8 and 9, inter alia, and Hansen Declaration, id. Without teaching, suggestion or motivation for such combination, such subject matter is not obvious.

In an objective sense, with hindsight, one might ask why create both the aglycon and the glycosyltransferase in the same cell, or more specifically, why put both pathways within the single yeast cell? Similarly, what would the skilled person think is the point of glycosylating an aglycon such as vanillin, just to deglycosylate it later, particularly if no art suggests such a combination? The surprising result, not taught, suggested or motivated in or from the cited art, is that this production process with both pathways within the single cell, which thereby involves both the initial production of the vanillin and its subsequent conversion into glycosylated vanillin together within the yeast cell, unexpectedly yields higher production, or an overproduction, of vanillin, as a result. Møller Declaration, para. 4, lines 7-15, and Hansen Declaration, *id.* I.e., there is a greater production ultimately of vanillin, both of the produced aglycon form and the glycosylated form of the aglycon vanillin as described in Applicants' developments. In the cited art, there is no teaching, suggestion, or motivation to instruct such high level production of the glycosylated version of the aglycon vanillin, as indicated in step (a) of Claim 1, and, particularly there is a complete lack of teaching, suggestion, or motivation to further consider a step (b) of Claim 1 in combination with step (a). Møller Declaration, para. 4, lines 12-13, inter alia, and Hansen Declaration, *id.* Parts of a combination separately known do not render the combination obvious without some teaching, suggestion or motivation toward the combination. See Declaration of Professor Birger Lindberg Møller, page 2, para. 4, line 13, and Hansen Declaration, *id.*

Without having the knowledge, provided by the present developments, that a single yeast cell producing both an aglycon together with a suitable glycosyltransferase is capable of producing higher amounts (overproduction) of the glycosylated vanillin, one skilled in the art would never even have considered developing a method of claim 1 hereof. Without this "overproduction" knowledge, one skilled in the art would have no incentive to develop a more complicated method, that would involve the step of first glycosylating the aglycon and then deglycosylating it to get the aglycon.

Moreover, none of the cited art includes both biosynthetic steps of synthesizing vanillin and synthesizing the glycosylated form of vanillin within a single yeast cell. Møller Declaration, paras. 4, 6, and 9, and Hansen Declaration, id. None of the cited art teaches, suggests or motivates the simultaneous synthesis of an aglycon, a suitable glycosyltransferase and thus the glycosylated form of the aglycon within the same yeast cell. The Office Action fails to point out any cited art that discloses genes encoding for the biosynthetic pathway, in the same yeast cell, for production of both the aglycon vanillin and the corresponding glycosylated form of the aglycon vanillin.

Moehs fails to teach these elements of Applicant's claimed development. Rather, Moehs only states the obvious, that a glycosylated form of solanidine may be made when the relevant glycosyltransferase (there, SGT) is present. See Affidavit of Professor Birger Lindberg Møller, para. 6, and Hansen Declaration, id. However, this glycosylated formation occurs outside the cell in Moehs. Møller Declaration, para. 6, lines 6-8, and Hansen Declaration, id. There is no second genetic pathway described within Moehs for the production of both the aglycon and the glycosyltransferase within the cell. Møller Declaration, para. 6, lines 3-6, and Hansen Declaration, id.

Moreover, with respect to what Moehs actually teaches and suggests, the present Final Office Action, page 4, states that, in Moehs:

“Figure 7 shows that solanidine was recovered. It also shows that the cell is capable of producing higher amounts of glycosylated solanidine with SGT present, than without. However, Moehs et al does not teach deglycosylating the aglycon, or that the aglycon is vanillin.” (emphasis added).

This underlined sentence from the Office Action represents a fundamental technical misinterpretation of the teachings of Moehs. See Declaration of Professor Birger Lindberg Møller, pages 3-4, para. 8-9, and Hansen Declaration, id.

As Applicants have previously explained in paragraph [0014] of Applicants' specification, the solanidine glucosyltransferase (SGT) in Moehs was cloned by using a plate based positive selection based on the higher toxicity of steroidal alkaloid aglycons relative to their corresponding glycosylated forms. Møller, para. 5, and Hansen, id. The Moehs cloning plate assay was based on the premise that the solanidine aglycon compound is toxic to the yeast cells used for the cloning. Møller, para. 5, and Hansen, id. Accordingly, a yeast cell expressing the cloned solanidine glucosyltransferase (SGT) will simply grow faster on a PLATE containing exogenously added solanidine. In short, in the plate assay, the yeast clones expressing the SGT will be able to glycosylate the "toxic" solanidine aglycon compound added to the plate, thereby rendering it less "toxic," and thus simply will be able to grow because there is less of the "toxic" solanidine aglycon compound to inhibit their growth. See Declaration of Professor Birger Lindberg Møller, page 3, para. 7, lines 4-6, and Hansen Declaration, id.

Thus, Moehs completely fails to teach that the yeast or E.coli have a biosynthetic pathway to produce the aglycon solanidine or the glycosylated form of the solanidine. See Affidavit of Professor Birger Lindberg Møller, page 3, para. 6, and Hansen Declaration, id. No relevant biosynthesis pathway genes for solanidine or any other aglycon are introduced in the yeast cells or E.coli cells described in Moehs. Combining both biosynthesis of the aglycon and its subsequent glycosylation to produce a non-toxic aglycon means that the toxic aglycon does not accumulate either in the medium or within the yeast cells. Thus the problem of growth inhibition observed in Moehs is not encountered by the approach presented in the current application. The application of the cloned SGT brought forward by Moehs is summarized in the last sentence of the introduction: "The molecular cloning of SGT opens the possibility of developing novel methods to decrease SGA levels in potato cultivars by down-regulating the expression of this enzyme using antisense RNA transgenes" (end of citation). Moehs thus advises downregulating SGT expression to reduce the level of the accumulated glucoside. This advice is in opposition to the approach of the instant application which teaches introduction of genes encoding all enzymes of a biosynthetic pathway for an aglycon concomitant with introduction of a glucosyltransferase

to convert the aglycon formed into the corresponding non-toxic glucoside with the aim of increasing the level of a desired glucoside.

Paragraph [0014] of Applicants' published application comments on Moehs:

[0014] Moehs, CP et al, Plant Journal (1997) 11:227-236 describes that a cDNA encoding a solanidine glucosyltransferase (SGT) was isolated from potato. The cDNA was selected from a yeast expression library using a positive selection based on the higher toxicity of steroidal alkaloid aglycons relatively to their corresponding glycosylated forms. The activity of the cloned SGT was tested in an *in vitro* assay based on isolated recombinant produced SGT.

Accordingly, Moehs achieves nothing more than the simple cloning of solanidine glucosyltransferase (SGT) as such. In Moehs, a cDNA yeast expression library containing the glucosyltransferase (SGT) was merely inserted into the yeast, and NOT the biosynthesis pathway for the aglycon compound solanidine. See Affidavit of Professor Birger Lindberg Møller, pages 2-3, para. 5, and Hansen Declaration, id.

The previous Office Action also relied on Figure 7 of Moehs to support an allegation that 'solanidine was recovered'. As previously argued by Applicants, Figure 7 is "simply" testing *in vitro* the activity of the cloned SGT. For the *in vitro* test, the recombinantly produced solanidine glucosyltransferase SGT is simply isolated and then tested for the correct activity *in vitro*. Møller Declaration, para. 8, and Hansen Declaration, id.

It should also be noted that Moehs actually teaches away from the present development. The glycosylation as taught in Moehs is for the particular purpose of rendering the solasodine less toxic for increased yeast cell growth; and thus, any deglycosylation would reverse this advantage and would thus be taught against, i.e., contra-indicated. Møller Declaration, para. 7, and Hansen Declaration, id. Teaching away such as this renders the current claimed subject matter non-obvious.

The rest of the cited art in the Office Action essentially states that the cited art teaches how to perform the deglycosylating of the aglycon step as such – e.g. by use of a beta-glucosidase as in the amended claim 21 of the present application. Though it may be true, *arguendo*, that one skilled in the art may know that by use of e.g. a beta-glucosidase, one can effect the deglycosylating of the aglycon step as such. However, Applicants claim more than mere glycosylating; it is rather the combination which provides the overproduction which is clearly not obvious over Moehs, in and of itself, or in any combination. Thus, the additional references cited by the Office Action for mere glycosylation, outside the cell, or deglycosylation in general, may not be combined with Moehs to support the current Section 103(a) rejections. None of the cited art teaches use of an inactivated gene in a yeast cell to obtain the results of Applicants' claimed development. The Preifert reference uses an *aat*-knockout mutant of *Pseudomonas*. See Priefert, p. 302, col. 1, line 11. This bacteria, which is not a yeast cell, lacks the relevant gene, as the gene has been deleted. This is in contrast to Applicants' development, where the relevant gene in the yeast cell has simply been inactivated. Thus, the reliance on Day and/or Priefert is mis-placed. See also, Møller Declaration, para. 10, and Hansen Declaration, id.

Still furthermore, with respect to the rejection based on Arend, Applicants respectfully submit that the application has already discussed and distinguished over Arend. As stated in paragraph [0013] of the publication version of the subject application:

[0013] Arend, J et al., Biotech. & Bioeng (2001) 78:126-131 and WO01/07631 describes cloning of a glucosyltransferase from the plant *Rauvolfia serpentina*. The cloned glucosyltransferase was inserted into *E. coli* bacteria. When the aglucones hydroquinone, vanillin and p-hydroxyacetophenone were added to the medium of cultivated cells of the engineered *E. coli*, the corresponding glucosides, arbutin, vanillin-D-glucoside and picein were synthesized. They also were released from the cells into the surrounding medium. (Emphasis added.)

Similar to Applicants' arguments regarding the Moehs reference, Arend merely and only teaches that a relevant glucosyltransferase may add glucose to an aglycon such as vanillin when that aglycon is added to a medium. This is unlike to Applicants' present development, where the

vanillin is synthesized inside a cell rather than added to a medium. Moreover, Arend does not teach the same in-cell biosynthetic pathways taught by Applicants. See Affidavit of Professor Birger Lindberg Møller, page 4, paras. 11-12, and Hansen Declaration, id.

For these reasons, the subject matter of Applicants' claims is not obvious over Moehs, Day, Arend, or Priefert, or any combination thereof. No matter what Arend, Day or Priefert might suggest regarding either glycosylating or deglycosylating an aglycon, or regarding the specific features of the aglycon, none of these references, alone or in any combination, cures the failure of Moehs to disclose the dual in-cell biosynthetic pathways leading to the overproduction of an aglycon of interest, namely, vanillin, in a microorganism, namely, a yeast cell. Thus, Moehs in combination with Arend, Day or Priefert or Arend, or any combination thereof, all fail to render claim 1 obvious or unpatentable. See Affidavit of Professor Birger Lindberg Møller, page 4, para. 10, and Hansen Declaration, id. Reconsideration and withdrawal of all of these obviousness rejections are thus also respectfully requested.

Lastly, Applicants respectfully offer the further considerations of the high degree of acceptance and acknowledgement by and further accolades received from others skilled in the art as a result of the publication of the advancements defined by the claims and specification hereof. More particularly, as set forth in paragraph 14 of the Møller Declaration and the Hansen Declaration, the increased biosynthesis of vanillin in yeast is described in the publication de Novo Biosynthesis of Vanillin in Fission Yeast (*Schizosaccharomyces pombe*) and Baker's Yeast (*Saccharomyces cerevisiae*), in APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2009. Moreover, both of the respected journals, Nature and Science have reported favorably on these advancements; e.g., "[t]hese *de novo* pathways for vanillin synthesis in yeast represent the first examples of one-cell microbial generation of these valuable compounds from glucose," Nature Reviews, Vol. 7, May 2009; Møller Declaration, para. 15, lines 12-14, and Hansen Declaration, id.; and, in explaining that the glycosylated form is not toxic to the yeast, "allowing the yeast to hold more of the compound," Science News, May 23, 2009, Vol. 175, No. 11; Møller Declaration, para. 16, lines 4-5, and Hansen Declaration, id.

Response to Arguments Concerning Claim Rejections – 35 USC §103(a)

The Office Action of November 4, 2009, on page 7, states that the Applicants' arguments from its previous Response to Office Action have not been found persuasive for a number of reasons. Applicants respectfully submit that these arguments are indeed persuasive.

The Office Action states, on page 7, that "Applicants are not claiming how to introduce the complete biosynthesis pathway of vanillin into a yeast microorganism. They are teaching production of an aglycon." However, in light of the above discussion, it is clear that claim 1 specifically claims the dual biosynthesis pathways for both vanillin and the glycosyltransferase within a yeast microorganism yielding production of vanillin and glycosylated vanillin. Thus, the cited references of Moebs and Day become even less applicable to Applicants' developments.

The Office Action, on page 7, further states that Moebs teaches 'using yeast to glycosylate an aglycon'. However, Moebs glycosylates outside the cell, after production and excretion of the glycosyltransferase. Moebs does not teach, suggest, or motivate generation of the aglycon and the glycosyltransferase inside the cell, let alone the glycosylating of the aglycon vanillin also typically in the cell. Moreover, as discussed above, Moebs fails to specifically teach, suggest or motivate fermenting a yeast cell which comprises a gene encoding a product involved in the biosynthesis pathway leading to vanillin and a glycosyltransferase gene encoding a glycosyltransferase capable of glycosylating the vanillin produced. Finally, Moebs specifically fails to teach, suggest or motivate such a yeast cell producing both vanillin and the corresponding glycosylated form of vanillin, all within the yeast cell. See Affidavit of Professor Birger Lindberg Møller, page 3, para. 6, and Hansen Declaration, id.

The Office Action takes the position that “there is reason to believe that glycosylating other aglycons will be successful”. This is contrary to fact and the established law relating to chemical compounds. It should be noted that chemistry, being a generally unpredictable art, does not provide for generalized expectations of success in or from one class of compounds to another. See In re Papesch, 315 F.2d 381, 137 USPQ 43 (CCPA 1963); here, this means that any success Moehs may have had using a single gene encoding the glycosyltransferase, with glycosylating solanidine outside the cell does not, without more, teach or suggest any expectation of success with a different compound, vanillin, for example (note, this is the general basis for the need for data in chemistry cases, as it is typically not predictable that a particular success is or may have been achieved, generally); and, particularly not when Applicants include the dual in-cell biosynthetic pathways.

Applicant therefore respectfully requests reconsideration and withdrawal of all rejections and consequently re-examination and allowance of all claims pending in this application; namely claims 1, 2, 4, 7, 8, 19 and 21.

CONCLUSION

Applicant respectfully requests that all of the claims be re-examined. A timely Notice of Allowance is requested to be issued in this case. Applicants believe that no additional fees or petitions are due with this filing, apart from the fees for filing a Request for Continued Examination and for an extension of time. However, should any such fees or petitions be required, please consider this a request therefore and authorization to charge Deposit Account No. 02-2093 as necessary.

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Respectfully submitted,

/peterbscull/
Peter B. Scull, Registration No. 37,932
Attorney for Applicants
USPTO Customer No. 43,439

BERENBAUM WEINSHIENK PC
370 Seventeenth Street, Suite 4800
Denver, Colorado 80202
Tel: 303-825-0800
Fax: 303-629-7610